

**EFFECTIVENESS OF SIX PLANT OILS ON THE PROTECTION OF
STORED COWPEA *VIGNA UNJUICULATA* AGAINST THE INFESTATION
OF COWPEA WEEVIL *CALLOSOBRUCHUS MACULATUS* (F.)**

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ABSTRACT

The effect of six plant oils of fenugreek, shallot, cabbage, juniper, frankincense and marjoram on the mortality, emerged adults and reduction in F1-progeny of cowpea weevil *Callosobruchus maculatus* (F.) on cowpea seeds was studied. Some biological aspects, persistence, germination and water absorption for treated cowpea seeds were also studied.

All tested oils at higher concentration 8 and 10 ml/kg caused a high level of mortality after 3 days of exposure and gave complete mortality (100%) after 7 days of exposure.

The high effect on the mortality was continued up to 8 weeks for the marjoram, frankincense and cabbage oils, while fenugreek and shallot deteriorated after 4 weeks and juniper had a moderate persistence. All oils were deteriorated after 10 weeks exposure period.

The Lc50 values of the five oils caused high reduction in the mean number of eggs laid and hatchability except frankincense had a moderate reduction (46.2%). On the other hand at the Lc99, no eggs laid for all oils.

The percentage of water absorption in both initial time and after 10 weeks was increased at three times (one, 5 and 24hrs) whoever was found. A slight difference between the treated and untreated cowpea seeds was found in both times.

No effect of these oils on the percentage of the seed germination was detected.

INTRODUCTION

The wide spread use of chemical insecticides and fumigation has caused some serious problems *i.e.* including development of insect resistant strains, toxic residues on stored cereals, pollution environment, ozone depleting and health hazards to seed retailers. It is necessary to find out more selective and safe materials which might fulfill this requirement.

There is renewed interest in using natural products to control insects in many agricultural environments, including post-harvest ecosystems. An example of natural products is plant oils, which different kinds, plant extracts and dusts. Also, using of plant products as oils, dusts and extracts are a new trend preserves the environment from contamination with harmful toxicants.

Carlos and Cardons (1981) reported that, cooking oil can penetrate minute opening of egg surface and stop the biological activity of the insects at any stage of development. The effectiveness of vegetable oil as protectants of pulses against infestation by *C. maculatus* was also investigated by Schoonhoven (1978), Zewar (1987) and El-zemity *et al.* (2002).

Currently, few chemicals are available for the use as fumigants that meet all the constraints. Methyl bromide, the most effective fumigant, will soon be restricted due to its potential ozone depleting properties (WMO, 1991). Moreover, it is highly toxic to warm blooded animals including humans (Dansi *et al.*, 1984). Phosphine fumigation, which was widely used, may also become increasingly limited in use due to resistance of stored – grain insects to phosphine that has now been reported in more than 45 countries (Bell and Willson, 1995, Chaudhry, 1995). In addition, phosphine has been argued to be genotoxic to occupationally exposed fumigators (Garry *et al.*, 1989). Because of increasing drawbacks of today's conventional compounds to replace those currently used.

Essential oils have low toxicity to warm blooded animals, high volatility, and are toxic to stored grain insects (Shaaya *et al.*, 1991, 1997).

The bioactivity of several plant oils, extracts and plant dusts as pest control agents against stored products pest was studied by many investigators Su 1989, Makanjuola 1989, Ivbijaro 1990, El-lakwah, *et al.* 1993, Tounsend 1997. Salwa 2000, Abdel- Aziz, 2002.

The present work aims to study the efficiency of six plant oils on cowpea seeds against *Callosobruchus maculatus* (F.). Also to evaluate the residual effect of tested oils during storage.

MATERIALS AND METHODS

Test insect:

Pulse beetle adults *Callosobruchus maculatus* F. obtained from a standard culture reared in the Stored Grain Insects Res. Dep. Lap., Plant Protection Institute, Agric. Research Center. These adults were reared on cowpea seed, *Vigna unguiculata* in an incubator maintained at $28\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ R.H. All experiments were also performed under these conditions.

Tested oils:

Six Plant oils of fenugreek, shallot, chabage, juniper, frankincense and marjoram were selected for this study. These oils were obtained from the local market.

Toxicity test:

Five samples of 20 gram each of cowpea seeds each was thoroughly mixed well with one of the concentrations 2, 4, 6, 8 and 10 ml/ kg of the tested oils. The treated seeds of each concentration were transferred into a clean glass jars (5cm diameter x 7.9 cm high), covered with muslin and sealed with a rubber band then left for 72hrs at room temperature for oil adsorption. Directly, after this period, twenty of newly emerged *C. maculatus* adults (>24hrs old) were kindly introduced into each jar then covered and sealed as usual and kept at $28 \pm 2^{\circ}$ and $65 \pm 5\%$ R.H. Each concentration was represented with three jars as replicates. In the same time, similar three jars contained adults and untreated seeds, as a control, were kept at the same conditions. Mortality counts of the adults were recorded at 1,2,3,5 and 7 days post-treatment and corrected according to Abbott's formula (1925).

These findings were statistically computed according to Finney (1952) to produce LC_{50} and LC_{99} and toxicity slope for each oil. No. of emerged adults and the reduction in F_1 - progeny were calculated after 5 weeks following treatment according to the following equation.

$$\% \text{Reduction} = \frac{\text{No. of emerged adults in control} - \text{No. of emerged adults in treatment}}{\text{No. of emerged adults in control}} \times 100$$

Effect on the deposited eggs, hatchability and progeny of adults:

Five grams of cowpea seeds treated with LC_{50} or LC_{99} level were placed in a suitable glass tube. Four couples of newly emerged adults were placed in each tube and covered with muslin, three tubs (replicates) were used for each concentration. All dead insects after one week were discarded and the seeds were examined for counting all eggs laid. Hatchability of eggs was indicated when they turned white showing that larvae had penetrated the seeds. Infested seeds were kept till the emergence of offspring which was also counted.

Residual efficiency:

A sample of 500gm clean cowpea seeds was used for each oil at LC_{99} concentration. Mixing was manually done in one liter glass jar and kept at the laboratory condition. The obtained quantities(10gm) were divided into several groups each placed into glass vials. Each group consists of three replicates for different storage period. Each replicate was infested with 20 adults *C. maculatus* (>24h) old in addition to another three vials lab. condition ($28 \pm 2^{\circ}C$ and $65 \pm 5\%$ R.H.).

Mortality counts were recorded after 72 hrs and no noticed emergency adults F_1 - progeny after 5 weeks from each treatment for all oils.

Seed germination and water absorption:

Germination of seeds treated with the tested oils at the LC_{50} and LC_{99} levels and untreated seeds as control was observed in Petri dishes after one and 10 weeks of treatments.

Four dishes (9cm diameter) were lined with cotton wool and filter paper then 25 seeds were placed on the cotton. Germination was recorded after 7 days later (according to Anonymous 1966).

Treated and untreated seeds (2gm for each replicates) were weighed and submerged in water. Water absorption was measured after 1, 5 and 24 hr as percent increase in weight after drying the seeds, with paper towels (according to Yantai, and Wendel E. Burkholder, 1981).

Statistical analysis

Differences between means of the different treatments in all experiments were statistically analyzed using ANOVA.

RESULT AND DISCUSSION

Effect of the plant oils:

Results concerning effect of six plant oils, fenugreek, shallot, cabbage, juniper, frankincense and marjoram on mortality, no. of emerged adults and reduction in F1- progeny of *C. maculatus* (F.) are given in tables (1a and 1b).

Results showed that adult mortalities were increased with increasing concentration and exposure time. Adults were highly affected at the highest concentration (10 ml/kg) after 3 days of exposure, the six oils caused considerably high mortality (96.7, 88.3, 86.7, 91.6, 85 and 80%) after three days for fenugreek, shallot, cabbage, juniper, frankincense and marjoram oils respectively. A complete mortality (100%) for all oils was obtained after 7 days of exposure at the highest concentration 8 and 10 ml/kg.

No of adults were emerged with fenugreek, shallot, cabbage and juniper but few emerged in low concentrations (2) and (2,4,6,8 ml/kg) for frankincense and marjoram, respectively. Also, reduction in F1- progeny was much higher than mortality values at various concentrations for fenugreek, shallot, cabbage and frankincense but lower than mortality in low concentration in juniper and marjoram. These results agree with Mohamed and Abdel- Aziz (2006) when used tooth- pick (*Ammi visnaga* L.) seed extract against *C. maculatus* (F.).

Table (2) showed that, fenugreek oil on the base of LC_{50} and LC_{99} was the most effective against this pest, 3.91 and 17.81 ml/kg, respectively.

The slope of toxicity lines indicated that, the highest insect sensitivity was exhibited by shallot oil (2.94) than the other oils. Similar results were reported by Afifi *et al.*, (1989). on *Sitophilus Oryzae*. On the other hand, the least sensitivity was by frankincense oil (3.76).

Data in table (3) indicated that, exposing *C. maculatus* adults for a week to any the tested oils reduced the number of eggs laid; 17, 20.3, 21, 33.3, 44.3 and 55 eggs / 2 females for shallot, fenugreek, marjoram, cabbage, juniper and frankincense oils, respectively at the LC_{50} compared with 102.3 eggs/2 females of the control. This reduction was calculated as 83.4, 80.2, 79.5, 67.5, 56.7 and 46.2% opposite these oils, respectively. The complete reduction in eggs laid (100%) was recorded when the seeds were treated with LC_{99} of any oil.

Table (1- a): Toxicity of three plant oils on *C. maculatus* (F.) adults.

Treatment oils	Concentration ml/Kg	%Mortality after indicated period (days)					No. of emerged adults	Reduction in progeny (%)
		1	2	3	5	7		
Fenugreek	2	0.0	5.0 ± 2.9 c	20.0 ± 2.9 d	45.0 ± 5.8 c	81.7 ± 4.4 a	0.0	100
	4	0.0	11.7 ± 1.7 d	40.0 ± 2.9 c	73.3 ± 7.3 b	91.7 ± 3.3 a	0.0	100
	6	8.3 ± 1.7 b	28.3 ± 1.7 c	75.0 ± 5.8 b	91.7 ± 1.7 a	100 a	0.0	100
	8	15.0 ± 2.9 b	50.0 ± 2.9 b	90.0 ± 2.9 a	98.6 ± 1.7 a	100 a	0.0	100
	10	45.0 ± 5.8 a	61.7 ± 1.7 a	96.7 ± 1.7 a	100 a	100 a	0.0	100
L.S.D at 5%		9.3	6.9	10.9	13.3	No significant		-
Shallot	2	0 c	5.0 ± 2.9 c	15 ± 2.9 e	40 ± 2.9 c	65.0 ± 5.8 c	0.0	100
	4	1.6 ± 3.3 c	13.3 ± 3.3 c	35 ± 2.9 d	71.7 ± 4.4 b	85.0 ± 2.9 b	0.0	100
	6	6.7 ± 3.3 c	20.0 ± 20.9 c	53.3 ± 4.4 c	86.7 ± 4.4 a	95.0 ± 2.9 a	0.0	100
	8	23.3 ± 4.4 b	46.7 ± 4.4 b	73.3 ± 4.4 b	93.3 ± 4.4 a	100 a	0.0	100
	10	35.0 ± 5.8 a	66.7 ± 4.4 a	88.3 ± 1.7 a	96.7 ± 1.7 a	100 a	0.0	100
L.S.D at 5%		11.4	11.4	10.6	11.5	9.8		
Cabbage	2	1.6 ± 3.3 c	11.7 ± 1.7 c	18.3 ± 3.3 d	35.0 ± 5.8 d	66.7 ± 4.4 d	0.0	100
	4	3.3 ± 3.3 c	10.0 ± 2.9 c	36.7 ± 1.7 c	58.3 ± 4.4 c	76.7 ± 4.4 c	0.0	100
	6	8.3 ± 1.7 bc	15.0 ± 2.9 c	63.3 ± 1.7 b	76.7 ± 4.4 b	90.0 ± 2.9 b	0.0	100
	8	15 ± 2.9 b	40.0 ± 2.9 b	78.3 ± 3.3 a	90.0 ± 2.9 a	100 a	0.0	100
	10	31.7 ± 4.4 a	55.0 ± 2.9 a	86.7 ± 1.7 a	96.7 ± 1.7 a	100 a	0.0	100
L.S.D at 5%		9.3	8.4	8.7	13.9	9.6		-

Table (1- b): Toxicity of three plant oils on *C. maculatus* (F.) adults.

Treatment oils	Concentration ml/Kg	Mortality after indicated period (days)					No. of emerged adults	Reduction in progeny (%)
		1	2	3	5	7		
Juniper	2	5.0±2.9 c	10.0±2.9 c	15.0±2.9 e	50.0±2.9 c	80.0±2.9 b	0.0	100
	4	1.6±3.3 c	10.0±2.9 c	33.3±4.4 d	80.0±2.9 b	95.0±2.9 a	0.0	100
	6	6.7±2.8 c	13.3±1.7 c	35.0±5.8 c	86.7±1.7 b	98.3±1.7 a	0.0	100
	8	8.3±1.7 b	30.0±2.9 b	75.0±2.9 b	95.0±2.9 a	100 a	0.0	100
	10	16.7±1.7a	43.3±1.7 a	91.6±1.7 a	100 a	100 a	0.0	100
L.S.D at 5%		6.1	7.7	11.8	7.3	6.1		
Frankincense	2	1.7±1.7 a	1.6±1.7 e	8.3±1.7 e	61.7±7.3 b	85.0±2.9 b	4	98.3
	4	3.3±1.7 a	11.7±1.7 d	21.7±4.4 d	90.0±2.9 a	96.6±1.7 a	0.0	100
	6	3.3±3.3 a	30±2.9 c	53.3±3.3 c	98.3±1.7 a	100 a		100
	8	1.7±1.7 a	40±2.9 b	75±2.9 b	100 a	-		100
	10	5±2.9 a	68.3±4.4 a	85±2.9 a	100 a	-	0.0	100
L.S.D at 5%		7.3	8.9	9.8	12.1	4.6		
Marjoram	2	0.0	0.0	13.3±1.7 e	45.0±7.6 c	81.6±1.7	75	68.8
	4	0.0	0.0	15.0±00 e	83.3±4.4 b	100 a	47.3	80.3
	6	0.0	11.7±4.4 c	40.0±2.9 c	95.0±2.9 a	100 a	20.3	91.5
	8	3.3+b	23.3±1.7 b	66.7±4.4 b	96.6±1.7 a	100 a	8	96.7
	10	10.0±2.9 a	35±2.9 a	80.0±2.9 a	100 a	-	0.0	100
L.S.D at 5%		6.6	8.9	8.7	15.0	6.1		
Control	-	-	-	-	-	-	240	--

Table (2): Lethal concentration (LC_{50} and LC_{99}) and slope of the plant oils after 3 days from treatment of *C. maculatus* (F.) adults at $28 \pm 2^\circ C$ and $65 \pm 5\% R.H$

Oils	LC_{50} (ml/kg)	LC_{99} (ml/kg)	slope
Fenugreek	3.91	17.81	3.536
Shallot	4.96	28.60	2.943
Cabbage	4.56	28.13	3.057
Juniper	5.31	29.26	3.139
Frankincense	5.57	23.17	3.756
Marjoram	6.29	36.06	3.067

Table (3): Effect of cowpea seeds treatment with six plant oils, at LC_{50} and LC_{99} on some biological aspects of *C. maculatus* (F.) adult at $28 \pm 2^\circ C$ and $65 \pm 5\% R.H.$

oils	Concentration ml/kg	Mean no. of egg 2/2% pairs		Hatchability	Mean no of F1-progeny	
		Mean number	Reduction %		Mean number	Reduction %
Fenugreek	LC_{50}	20.3 ± 3.9	80.2	50	-	100
	LC_{99}	0.0	100	-	-	100
Shallot	LC_{50}	17.0 ± 3.2	83.4	46.3	1 ± 0.0	98.0
	LC_{99}	0.0	100	-	-	100
Cabbage	LC_{50}	33.3 ± 3.3	67.5	51.7	-	100
	LC_{99}	0.0	100	-	-	100
Juniper	LC_{50}	44.3 ± 6.6	56.7	56.3	-	100
	LC_{99}	0.0	100	-	-	100
Frankincense	LC_{50}	55 ± 9.0	46.2	61.0	9.3 ± 1.9	84.4
	LC_{99}	0.0	100	-	-	100
Marjoram	LC_{50}	21.0 ± 3.6	79.5	48.0	-	100
	LC_{99}	0.0	100	-	-	-
control	-	102.3 ± 3.9	-	89.7	59.7 ± 3.5	

Also, a reduction in the eggs hatching occurred at LC_{50} for these oils; 50, 46.3, 51.7, 56.3, 61 and 48.0% respectively, compared with control (89.7%).

As for the insect progeny it severely affected, and no progeny was gained at LC_{50} and LC_{99} in case of fenugreek, cabbage, juniper and marjoram, but a few numbers appeared and 9.3 individual at LC_{50} only for shallot and frankincense oils.

Results in table (4) indicated that, marjoram, frankincense and cabbage were the most persistent oils, these gave mortality 75, 65 and 65% till the 8 weeks compared with the fenugreek and shallot(40 and 45) at the same time. But juniper was moderate persistent after the same time (60%).

Table (4): Corrected mortality percentage *C. maculatus* adults after 3 days of exposure to cowpea seeds treated with Lc_{50} of plant oils and kept for different period under insectary condition.

Period (weeks)	Mortality (%) after indicated period (weeks)					
	Fenugreek	shallot	cabbage	Juniper	Frankincense	Marjoram
Initial	100	100	100	100	100	100
2	85	85	90	100	100	100
4	75	80	80	85	90	95
6	60	75	70	80	85	90
8	40	45	65	60	65	75
10	0	15	25	20	30	35

These results in table (3 and 4) agree with those reported by Salwa and Shereef (2000).

Data presented in table (5) indicated that, the percentage of water absorption at the initial time not varied consistently with the amount of tested oils applied. In addition, differences between concentrations or between oils were relatively small except juniper oil is high effect on seed treatment in water absorption. But the end of storage after 10weeks, the water absorption was slight decreased compared with the initial treatment of these oils at Lc_{50} and Lc_{99} . These results agree with those reported by Salwa and Shereef 2000.

Table (5): Water absorption percentages at LC_{50} and Lc_{99} of cowpea seeds treated with different oils at the initial and end period of storage.

Oils	LC 50			LC 99		
	Water absorption %			Water absorption %		
	Initial					
	1 hr	5 hrs	24 hrs	1 hr	5 hrs	24 hrs
Fenugreek	55.7 ± 2.6	175.3 ± 2.0	213.7 ± 3.2	52.7 ± 4.5	185 ± 4.0	206.7 ± 4.4
Shallot	53.7 ± 5.4	187.3 ± 2.4	211.3 ± 0.9	44 ± 7.6	156 ± 9.1	204.3 ± 0.7
Cabbage	61 ± 0.6	188 ± 3.1	217.7 ± 2.4	50.7 ± 3.9	177 ± 4.4	207 ± 1.8
Juniper	51.3 ± 2.3	186.3 ± 1.8	218.7 ± 2.4	37.7 ± 3.4	136.3 ± 4.9	204 ± 3.1
Frankincense	63.3 ± 3.2	193 ± 2.1	220.7 ± 2.0	59.3 ± 3.7	195.3 ± 2.9	209.7 ± 2.3
Marjoram	65.3 ± 4.8	193.7 ± 1.3	218.7 ± 2.9	51.7 ± 4.1	163.7 ± 7.9	205 ± 5.9
Control	67.3 ± 7.7	186.3 ± 2.3	215.3 ± 1.2	67.3 ± 7.7	186.3 ± 2.3	215.3 ± 1.2
	End period of storage					
Fenugreek	50 ± 2.1	165 ± 2.3	201.7 ± 2.4	45.7 ± 2.4	170 ± 9.1	195 ± 2.9
Shallot	45.3 ± 2.0	174.7 ± 4.3	202.7 ± 2.2	40.3 ± 2.4	151 ± 4.4	192 ± 3.4
Cabbage	57 ± 3.1	175.7 ± 4.1	209 ± 1.0	46 ± 2.0	129 ± 4.4	195.3 ± 0.7
Juniper	50 ± 0.6	177.3 ± 2.3	210 ± 3.1	35.3 ± 2.3	169.7 ± 3.4	191.7 ± 1.8
Frankincense	59.3 ± 3.2	185.7 ± 3.4	213.7 ± 1.9	53 ± 4.8	183 ± 2.4	198.7 ± 2.3
Marjoram	55.3 ± 2.3	177 ± 1.5	211 ± 1.2	62 ± 3.4	152 ± 2.9	189.3 ± 3.4
Control	65 ± 4.4	181.3 ± 2.1	209 ± 0.6	65 ± 4.4	181.3 ± 2.1	209 ± 0.6

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Table (6) shows that, the viability of cowpea seeds slightly affected by all tested oils. A reduction in germination rate was observed when the LC_{99} at initial and end storage was tested. The highest reductions in germination percentages were (20,20, 16, and 16) for marjoram, frankincense, juniper and shallot, respectively, at the end of storage and at LC_{99} levels.

These results agree with EL-Kashlan (2004),he found that no effect of four natural plant oils on germination of cowpea seeds.

Table (6): Effect of the LC_{50} and LC_{99} levels of plant oils on the percent of cowpea seeds germination at the initial and the end period of storage under insectary condition.

Oils	Initial time			End period storage	
	Concen. ml/kg	Germination	Reduction	Germination	Reduction
Fenugreek	LC_{50}	80	4	80	4
	LC_{99}	81.3	2.7	80	4
Shallot	LC_{50}	78	6	76	8
	LC_{99}	72	12	68	16
Cabbage	LC_{50}	78	6	84	0.0
	LC_{99}	72	12	76	8
Juniper	LC_{50}	72	12	76	8
	LC_{99}	68	16	68	16
Frankincense	LC_{50}	72	12	68	16
	LC_{99}	70	14	64	20
Marjoram	LC_{50}	72	12	76	8
	LC_{99}	68	16	64	20
control		84	-	84	-

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تأثير ستة أنواع من الزيوت النباتية لوقاية بذور اللوبيا من الإصابة بحشرة خنفساء اللوبيا *Callosobruchus maculatus*

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تم اختبار فعالية زيوت ستة نباتات هي (الحلبة، الكرات، الكرنب، العرعر، اللبان والبردقوش) على نسب الموت ونسب خروج حشرات خنفساء اللوبيا وكذلك الخفض في تعداد الجيل الأول لها عند تربيتها على بذور اللوبيا المعاملة بهذه الزيوت. تم أيضا دراسة تأثير هذه الزيوت على بعض الصفات البيولوجية للحشرة وكذلك مدى ثبات تأثيرها على نسبة انبات البذور ومعدل امتصاصها للماء. أوضحت النتائج ما يلي:

- أعطت كل الزيوت المختبرة عند التركيزات (٨، ١٠ ملي/كجم) نسب موت عالية بعد ثلاثة أيام (٩١،٦-٨٠، ٩٠،٠-٦٦،٧) وبلغت ١٠٠% بعد سبعة أيام من التعريض.
- أوضحت النتائج كذلك أن التأثير الباقي لزيوت البردقوش واللبان والكرنب قد أعطت نسبة موت عالية نسبيا (٦٥-٧٥%) لمدة ثمانية أسابيع بينما زيوت الحلبة والكرات لمدة أربعة أسابيع فقط (٧٥-٨٠%) بينما كان تأثير زيت العرعر فوق المتوسط (٦٠%) بوقد انتهى تأثير كل الزيوت تقريبا بعد ١٠ أسابيع.
- سببت قيم LC_{50} لخمسة زيوت انخفاض شديد في عدد البيض (٥٦،٧-٨٣،٤%) ما عدا زيت اللبان حيث كان تأثيره على الانخفاض متوسطا (٤٦،٢%) هذا وقد وصل الخفض في إعداد البيض إلى ١٠٠% عند استعمال أى من الزيوت بتركيز LC_{99} .
- بينما كان تأثير هذه الزيوت على نسبة الإنبات لبذور اللوبيا بتركيزات LC_{50} ، LC_{99} قليلا أو ضعيفا وكذلك على معدل امتصاصها للماء.